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(पहला पुनरीक्षण)

Method for Estimation of Total Dietary Fibre in Foodstuffs

(First Revision)

ICS 67.050

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भारतीय मानक ब्यूरो
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Test Methods for Food Products Sectional Committee, FAD 28

FOREWORD

This Indian Standard (First Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Test Methods for Food Products Sectional Committee had been approved by the Food and Agriculture Divisional Council.

Dietary fibre (DF) is the sum of the polysaccharides and lignin present in the human diets which are not digested by the endogenous secretions of the upper gastro-intestinal tract. It may also include other non-digestible polysaccharides, such as gums and mucilages as well as other plant cell wall components, for example, non-lignin phenols, cutin, phytic acid, ester-linked acetic acid and so on. By definition, dietary fibre consists of the remnants of plant cells, polysaccharides, lignin and associated substances resistant to hydrolysis (digestion) by the alimentary enzymes of humans. Based on the solubility in water the dietary fibre is classified into insoluble dietary fibre (IDF) or less fermentable fibre and soluble dietary fibre (SDF) or fermentable fibre.

The different methods of analysis of food fibres can broadly be classified as those based on direct carbohydrate determination and on gravimetric determination. The present standard method of dietary fibre analysis in foods is adopted based on similarity to the human digestive process. Hence, enzymatic and gravimetric method (AOAC - Association of Official Analytical Chemists) is widely used for the determination of insoluble, soluble and total dietary fibre in foods.

In reporting the result of a test or analysis made in accordance with this standard, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

METHOD FOR ESTIMATION OF TOTAL DIETARY FIBRE IN FOODSTUFFS

(First Revision)

1 SCOPE

This standard specifies an enzymatic and gravimetric method for determination of insoluble, soluble and total dietary fibre after the enzymatic digestion of carbohydrate, protein and starch in foodstuffs.

NOTE — Sample should be analyzed in duplicate along with reagent blank.

2 REFERENCES

The following standard contains provisions which through reference in this text, constitute provisions of this standard. At the time of publication, the edition indicated was valid. Standard is subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below:

IS No.	*Title*
1070 : 1992	Reagent grade water (*third revision*)

3 PRINCIPLE

3.1 The moisture and fat free sample of food is first gelatinized at 95°C, then subjected to heat stable α-amylase followed by protease and finally with amyloglucosidase incubation to hydrolyze carbohydrate, protein and starch. The defatted sample is gelatinized at 95–100ºC and digested with heat stable α-amylase, followed by protease and amyloglucosidase enzymes to remove starch and protein under carefully controlled conditions. Dietary fibre is the loss of weight resulting from ashing, after corrected for protein and reagent blank.

3.1.1 *Insoluble Dietary Fibre (IDF)*

After enzymatic treatment, filtered insoluble residue (the fraction remaining after digestion) from sample is analyzed for protein and the residue from the duplicate sample for ash. IDF value is obtained after correcting for protein and ash content of residue.

3.1.2 *Soluble Dietary Fibre (SDF)*

Soluble dietary fibre is precipitated from the filtrate obtained in the above step with the addition of four volumes of 95 percent ethanol. Filtered residue from one sample is analyzed for protein and ash from another residue. SDF value of the sample is weight of the residue corrected for the weight of protein and ash.

3.1.3 *Total Dietary Fibre (TDF)*

The total dietary fibre content of the food sample is the sum of the insoluble and soluble fractions after subtracting the reagent blank. This value can be expressed as grams per 100 g of the dry weight or as it is basis as required for the original foodstuff (moisture should be taken care-off, if moisture free samples are taken).

4 EQUIPMENTS AND MATERIALS

4.1 Cyclone Mill

4.2 Analytical Balance (with sensitivity of ± 0.1 mg)

4.3 Water Bath, boiling

4.4 Hot Air Oven

4.5 *p*H Meter

4.6 Shaking Water Bath with Thermostat Temperature Control

4.7 Filtration Module (Vacuum Filter Unit)

4.8 Muffle Furnace

4.9 Glass Beakers (500 ml)

4.10 Aluminum Foil

4.11 Celite, Acid Washed

4.12 Fritted Crucibles or Filtering Crucibles

4.13 Desiccator

4.14 Vacuum Pump

4.15 Thermometer

4.16 Graduated Pipette

4.17 Pipette with Tips

5 REAGENTS AND ENZYMES

5.1 Quality of Reagents

Unless specified otherwise, pure chemicals shall be employed in tests and distilled water (*see* IS 1070) shall be used when the use of water as a reagent is intended.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the result of analysis.

5.2 0.08 M Phosphate Buffer *p*H 6.0

Dissolve 1.400 g of anhydrous Na2HPO4, and 8.400 g of anhydrous NaHPO4, approximately 700 ml distilled water, check *p*H and adjust if necessary with NaOH or H3PO4, and make up to 1 litre with distilled water, store in tightly capped container at room temperature.

5.3 0.275 M NaOH Solution

Carefully add 275 ml of 1.0N NaOH solution to 700 ml DW in one liter volumetric flask and make up to 1 litre with distilled water, store in a capped container at room temperature.

5.4 0.325 M HCl Solution

Carefully add 325 ml of 1.0 M HCl solution to 600 ml of distilled water in one litre volumetric flask and make up to 1litre with DW, store in a capped container at room temperature.

5.5 95 Percent Ethanol

5.6 78 Percent Ethanol

Take 207 ml of distilled water into a one liter volumetric flask and dilute to volume with 95 percent ethanol.

5.7 Acetone

5.8 Heat Stable Alpha Amylase (Sigma or Equivalent)

Heat stable α-amylase from *Bacillus licheniformis.*

5.8.1 *Storage*

Store at 4°C. At this temperature the enzyme remains active for one year. Test the enzymatic activity after six months from opening.

5.9 Protease (Sigma or Equivalent)

Freeze-dried enzyme from *Bacillus licheniformis*.

5.9.1 *Storage*

Store at 4°C. At this temperature the enzyme remains active for one year. Test the enzymatic activity after six months from opening.

5.10 Amyloglucosidase (Sigma or Equivalent)

Obtained from *Rhizopus* mold origin, having an activity of 5 000 - 10 000 units per gram solid. (add equivalent units of amyloglucosidase enzyme solution, if liquid).

5.10.1 *Storage*

Store at 4°C. At this temperature the enzyme remains active for one year. Test the enzymatic activity after six months from opening.

NOTE — One unit of amylase digests large chain carbohydrate into disaccharides in 15 min at *p*H 6.0 at 95°C.

a) *Based on Nelson/Somogyi Reducing Sugar with Soluble Starch as Substrate* — 10 000 ±1 000 units/mL (one unit is defined as the amount of enzyme required to release 1 µmole reducing sugar equivalents per minute at *p*H 6.5 and 40°C) (AOAC 991.43).

NOTES:

1 One unit of protease hydrolyses 1.0 mg of protein into amino acids in 30 min at *p*H 7.5 at 60°C.

2 One unit of amyloglucosidase hydrolyses the starch and disaccharides into glucose units in 30 min at *p*H 4.5 at 60°C.

b) *Based on Starch/Glucose Oxidase–peroxidase Method* — 2 000–3 300 units/mL (1 unit of enzyme activity is defined as the amount of enzyme required to release 1 **µ**mole glucose per minute at *p*H 4.5 and 40**°**C). (AOAC 991.43)

NOTE — 0.05M MES-TRIS buffer (*p*H 8.2, at 24°C) can be used instead of 0.08 M phosphate buffer (*p*H 6.0), wherein *p*H adjustment before addition of each enzymes can be omitted (as per AOAC 991.43).

6 PROCEDURE

6.1 Principle

a) $\text{Starch} + H_2O \xrightarrow{\alpha\text{-amylase + amyloglucosidase}} \text{D-glucose}$.

b) $\text{Protein} + H_2O \xrightarrow{\text{protease}} \text{peptides}$.

c) Dietary fibre determined gravimetrically following alcohol precipitation.

d) Ash and residual protein determined on DF residues and subtracted.

6.2 Preparation of Assay Sample

Cereals, pulses, oil seeds, spices and condiments, where fat content is more than 5 percent are first defatted using solvent ether extraction method. Fruits and vegetables are cut into small pieces and freeze dried. Freeze dried fruit samples are extracted with 80 percent ethanol to remove total soluble free sugars before milling. Milled samples are stored in a desiccator at room temperature, until taken for dietary fibre analysis. You also need to correct for sugar losses when removing sugar from freeze dried fruit samples. Powdered food sample is taken in duplicate for the estimation of dietary fibre analysis.

6.2.1 The sample taken for the assay shall be representative of the entire lot. The sample of food grains shall be milled to a particle size of 0.5 - 1 mm in a cyclone mill. The dry matter content of all the samples is determined as the weight after drying for 5 h at 105°C.

6.2.2 *Extraction of Lipids*

This step is recommended for foodstuffs containing more than 5 percent of lipids to facilitate the subsequent removal of free sugars by ethyl alcohol extraction. Extract the lipids by 5 h soxhlet extraction using chloroform methanol mixture or solvent ether. This system removes the free, bound and polar lipids. Estimate quantitatively the lipid content of the sample and proceed with the lipid free residue for further analysis.

6.2.3 *Removal of Free Sugars*

Take about 3 g moisture and lipid free sample in a glass centrifuge tube of 100 ml capacity. Add 50 ml of boiling 80 percent ethyl alcohol. Stir well and centrifuge at 2 000 rpm for 5 min. Discard the supernatant. Repeat the 80 percent ethyl alcohol treatment two more times.

NOTES:

1 If the free sugar content of the sample is to be estimated, then collect the ethyl alcohol extract quantitatively.

2 If the food sample contains less than 5 percent fat and free sugars, sample (moisture free) can be used directly for determination of dietary fibre.

6.2.4 *Preparation of Crucibles*

Clean the crucibles thoroughly, heat one hour at 525°C. Add 0.5g of celite to each crucible and dry at 130°C to constant weight (one hour or more). Cool in desiccator and weigh to nearest 0.1g and record the "celite + crucible weight. Store in a desiccator until needed for analysis.

6.3 Estimation of Dietary Fibre

6.3.1 Run reagent blank along with the samples. The contribution of any of the reagents to the residue is determined in the blank without sample and the measured blank value is to be deducted from the sample.

6.3.2 Weigh samples in quadruplicate (0.5 - 1.0 g) into 500 ml glass beakers and add 50 ml of 0.08M phosphate buffer (*p*H 6.0) to each beaker. Add 0.1 ml (500 units) öf heat stable α-amylase, cover the beakers with aluminum foil and place in a water bath maintained at 95°C for 15 min. Shake the beakers gently at 5 min. interval. Time of incubation should be 15 to 30 min.

6.3.3 Cool to room temperature and adjust *p*H to 7.5 with 10 ml of 0.275 M sodium hydroxide (NaOH) solution. Then add 5 mg of protease dissolved in 0.1 ml 0.08 M phosphate buffer (*p*H 6.0) to the solution. Cover the beakers with aluminum foil, incubate in shaking water bath maintained at 60°C for 30 min. Cool and adjust the *p*H to 4.5 with 10 ml of 0.205 M phosphoric acid (H_3PO_4) or 0.325N HCl solution. Then add 0.3 ml amyloglucosidase (1 700 units), cover the beakers with aluminum foil and incubate at 60°C for 30 min in a shaking water bath.

6.3.4 Filter the solution thus obtained through sintered glass crucibles (fibretec filtration module E 1027 or equivalent) containing celite. Transfer the precipitate is quantitatively from enzyme digest to crucible and wash with 20 ml of 78 percent ethanol and 10 ml of 95 percent ethanol and finally with 10 ml of acetone. Then take it out from the filtration module.

6.3.5 *IDF*

Dry the crucibles containing the residue overnight in hot air oven at 100°C, cool in a desiccator and weigh to determine weight of IDF residue.

Analyze a duplicate residue for protein (N × 6.25) by Kjeldahl method and incinerate another residue at 525°C in a muffle furnace for 5 h for ash.

6.3.6 *SDF*

To the left over filtrate (combined filtrate and washings), add four volumes of 95 percent ethanol to precipitate SDF. After one hour, transfer the precipitate into crucible containing celite, which is fitted in fibretec filtration module or equivalent.

Repeat the above procedure of estimating protein in sample residue and ash content is repeated in the duplicate sample residue to get SDF value. Like IDF value, correct the SDF values for protein and ash contents in the residue as mentioned above.

6.4 Calculations

6.4.1 *Blank (g)*

Weight of the blank residue (g) – [protein* in blank residue (g) + ash* in blank residue (g)]

Respective blank values are to be determined for IDF/SDF separately

6.4.2 *IDF (Percent)*

$$\frac{\text{IDF residue (g)} - [\text{protein in IDF residue (g)} + \text{ash in IDF residue (g)} + \text{Blank (g)}] \times 100}{\text{Weight of the sample taken (g)}}$$

6.4.3 *SDF (Percent)*

$$\frac{\text{SDF residue (g)} - [\text{protein in SDF residue (g)} + \text{ash in SDF residue (g)} + \text{Blank (g)}] \times 100}{\text{Weight of the sample taken (g)}}$$

6.4.4 *Total Dietary Fibre (TDF) (Percent) = IDF (Percent) + SDF (Percent)*

* Protein is due to enzymes and ash is due to the buffer

6.4.5 Express the results as total dietary fibre content as g/100 g on dry mass basis or as is basis as required after correcting for the moisture and lipid content.

Bibliographical References

1. AOAC (Methods 985.29, 991.42, 991.43 and 993.19)
2. AACC (Methods 32-05.01, 32-06.01, 32-07.01 and 32-21.01) and
3. CODEX (Type I Method)
4. Total Dietary Fibre Assay Kit, for the measurement and analysis of total, soluble and insoluble dietary fibre according to AOAC and AACC approved methods. See General Referee Reports: Journal of AOAC INTERNATIONAL, Vol. 81 No. 1, 1998.
5. Fibre is a mixture of complex organic substances, including hydrophilic compounds, such as soluble and insoluble polysaccharides and non-digestible oligosaccharides, as well as a range of non-swellable, more or less hydrophobic, compounds such as cutins, suberins and lignins. The procedures for the determination and analysis of total dietary fibre as outlined in our booklet are based on the methods of Lee et al.1 and Prosky et al. 2,3 (AOAC 991.43, AOAC 985.29, AACC 32-07.01 and AACC 32-05.01). However, the enzymes in the Megazyme Total Dietary Fibre Kit can also be used in other dietary fibre analytical methods such as AACC Method 32-21.01 and AACC Method 32-06.01.
6. Association of Official Analytical Chemists (1985). Official Methods of Analysis, 14th ed., 1st suppl. Secs. 43, A14-43, A20, p.399.
7. Association of Official Analytical Chemists (1986). Changes in methods. J. Assoc. Off. Anal. Chem., 69 : 370.
8. Association of Official Analytical Chemists (1987). Changes in methods. J. Assoc. Off. Anal. Chem., 70 : 393.

   Two separate methods are described in the associated data booklet:

   METHOD 1:
9. DETERMINATION OF TOTAL, SOLUBLE AND INSOLUBLE DIETARY FIBRE

   Based on AOAC Method 991.43 “Total, Soluble, and Insoluble Dietary Fibre in Foods” (First Action 1991) and AACC Method 32-07.01 “Determination of Soluble, Insoluble, and Total Dietary Fibre in Foods and Food Products” (Final Approval 10-16-91).

   METHOD 2:
10. DETERMINATION OF TOTAL DIETARY FIBRE

    Based on AACC method 32-05.01 and AOAC Method 985.29

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